REMARKS

I. Status of the Claims/Amendment

Claims 6-33 were previously withdrawn.

Claims 1, 2, and 5 have been amended. No new matter has been added.

Claims 1-5 are presently under examination.

II. Claims 1-5 – 35 U.S.C. 112, second paragraph

Claim 5 has been amended to recite, "the binding of said low-molecular weight compound to monoanionic phospholipid is independent of calcium". This amendment is supported in the Specification, pg. 7, ¶25, wherein a list of monoanionic phospholipids are provided to define the phospholipid species of interest, and at page 3, ¶05, where the term "low molecular weight fluorescent chemical" is provided. A "low molecular weight" compound is one that has a lower molecular weight relative to annexin V, annexin V being a protein with an approximate molecular weight of 35,000 Daltons (See Specification, pg. 3, ¶03). These further clarifying amendments render claim 5 limiting of the subject matter of claim 1, from which it depends.

The Action states that the "detecting" step of claim 1 appears indefinite and confusing, and appears to be inconsistent with the Ojeda *et al.* (JACS, 2002) reference. To even further clarify the intended meaning, claim 1 has been amended to recite, "detecting the presence of monoanionic phospholipids by fluorescence emission from said low molecular weight compound". This makes it clear that the low molecular weight compound having the chemical structure defined in the claim emits fluorescence when is bound to monoanionic phospholipid on a cell membrane/vesicle.

Claims 2-5, as dependent on claim 1, are similarly even more clarified.

Favorable consideration of the claims is respectfully requested.

III. Claims 1-5 – 35 U.S.C. 103(a) over Reutelingsperger (U.S. 5,834,196) taken in combination with Ojida *et al.* (JACS 2002, 6256-6258)

The Action raises a concern over the Reutelingsperger patent ('196), in combination with the Ojida *et al.* reference. Applicant respectfully traverses.

The present claims focus the use of a particular low-molecular weight group of compounds having the defined chemical structure (anthacene core, such as PSS-380) of claim 1. (See claim 1, Specification, pg. 4, ¶11; pg. 9, ¶39). This compound is not a protein, and therefore is unlike the annexin described in the cited '196 patent. Specifically, the cited Reutelingsperger patent relates to the use of a very high molecular weight compound, a protein, known as annexin, as the detectable reagent (See ABSTRACT). The Reutelingsperger '196 patent fails to make any suggestion or provide any teaching that a low molecular weight compound might be used for the same purpose. Therefore, this reference fails to have relevance to the presently claimed method, which utilizes a completely different, and much smaller, compound.

The Ojida *et al.* article does not relate to cell death or apoptosis, and does not suggest that the compounds identified therein, such as PSS-380, would be even remotely useful in detecting cell death or apoptosis. Moreover, the compounds therein, including PSS-380, are not described as useful for detecting monoanionic phosphate diester species of a phospholipid, such as those of phosphatidylserine on/within a cell membrane. This feature is now even more explicitly included within the pending claims.

The present inventors have provided a discovery that could therefore not have been even remotely predicted from the work reported in the cited '196 patent together with Ojida *et al.*

No basis has been stated that would support a suggestion to combine the '196 patent with the Ojida *et al.* article, or that would suggest substituting a non-protein, low molecular weight, reagent, such as PSS-380, in place of a protein, high molecular weight reagent, annexin V, in a method for detecting or monitoring cell death or apoptosis, or specifically for binding monoanionic phospholipids of a cell membrane or vesicle. Ojida *et al* does not provide any support of an expectation that these very different types of molecules, annexin and PSS-380, would be interchangeable. In fact, because the two references relate to entirely different classes of compounds altogether, a protein and a non-protein, chemical

sensor, it is highly unlikely that those of skill in the art would expect these compounds to be interchangeable in the methods of the other at all.

As noted in the instant application, Ojida *et al.* relates to a report that PSS-380 binds dianionic phosphorylated compounds, such as phosphotyrosine, in water. However, the present claims focus the finding that this low-molecular weight group of anthacene-based reagents, such as PSS-380, binds to monoanionic phosphate diesters and emits fluorescence, thus making it a useful detecting compound for monoanionic phospholipids.

Figure 1 shows the fluorescence emission spectrum for different samples with increasing phospholipid concentration. The y-axis is the Flourescence intensity in arbitrary unites (a.u.) given by the machine. The x-axis is the range of emission wavelengths that were measured.

Figure 2 shows the change in fluorescence intensity at one specific emission wavelength (440 nm). The y-axis shows I/Io which is the relative change in intensity, that is, the intensity (I) that is observed after addition of a certain concentration of phospholipid divided by fluorescence intensity when no phospholipid is present (Io).

Claim 1 defines two ways that the present low-molecular weight, non-protein compounds, particularly PSS-380, indicates the presence of anionic phospholipids:

The first is that the low-molecular weight compound binds to monoanionic phospholipids on the surface of vesicles, and thus the vesicles or cells can be visualized by fluorescence microscopy or counted by flow cytometry. The second is that the fluorescence emission spectrum of the compound changes in the presence of monoanionic phospholipids (as shown in Figures 1 and 2), and the changes in intensity at one or more wavelengths can be used to determine phospholipid concentration. Neither of these findings could have been derived from the teachings of the combination of references cited.

For the above reasons, among others, Applicant respectfully requests that the rejection be withdrawn, and that the claims be allowed to issue.

IV. Conclusion

In view of the foregoing, this application should be in condition for allowance. A notice to this effect is respectfully requested. Should the examiner have any questions, comments, or suggestions that would expedite the prosecution of the present application to allowance, Applicant's undersigned representative earnestly requests a telephone conference. Applicant's representative, Denise L. Mayfield, may be reached directly at (703) 563-2003. Applicant's representative also requests a telephone interview with the Examiner upon receipt of the present response.

Respectfully submitted,

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